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CALBIOCHEM®

Telomerase Inhibitor I

Cat. No. 581000

Size

10 mg

Synonym: 2,6-Bis[3-[(2-hydroxymethyl)-N-methylpiperidino]propionamido]-anthracene-9,10-dione, Diiodide

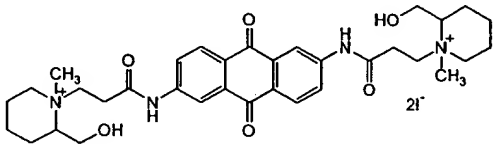
Description: A non-nucleoside 2,6-diamidoanthraquinone analog that appears to function as a low molecular weight mimic of K^+ in stabilizing the G-quadruplex folded structure, thus inhibiting telomerase activity (IC_{50} = 23 µM for human telomerase).

Form: Brown-orange solid

Molecular Weight: 860.6

Molecular Formula: $C_{34}H_{46}I_2N_4O_6$

Structure:



Purity: ≥85% by HPLC

Solubility: DMSO (200 mg/ml)

Storage: Freezer (-20°C). Protect from light. Following reconstitution aliquot and freeze (-20°C). This product is stable for 3 years as supplied. Stock solutions are stable for up to 3 months at -20°C.

Toxicity: MSDS available upon request.

Reference: Sun, D., et al. 1997. *J. Med. Chem.* **40,** 2113.

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Revised: 12-March-03

CALBIOCHEM®

Telomerase Inhibitor II

Cat. No. 581002

Size

50 nmol

Synonym: MI-2

Description: An antisense oligonucleotide that covers the template region of mouse telomerase RNA gene efficiently and inhibits telomerase activity at concentrations of less than 5 µM.

Form: Lyophilized solid. Packaged under an inert gas.

Molecular Weight: 5700

Sequence: 5′-d(ATGAAAATCAGGGTTAGG)-3′

Purity: ≥90% by HPLC

Solubility: H_2O

Storage: Deep-freeze (-70°C). Protect from light. Hygroscopic. This product is stable for 3 years as supplied.

Toxicity: MSDS available upon request.

Reference: Blasco, M.A., et al. 1995. *Science* **269,** 1267.

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Revised: 26-August-99

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Telomerase Inhibitor III

Cat. No. 581004

Size

150 nmol

Synonym: TAG-6

Description: A short hexameric phosphorothioate oligonucleotide (PS-ODN) telomer mimic that inhibits telomerase activity in cell lysates and lengthens cell doubling time *in vitro* and *in vivo* at concentrations of less than 2.5 µM.

Form: Lyophilized solid. Packaged under an inert gas.

Sequence: 5′-d(TTAGGG)-3′

Solubility: H_2O

Storage: Deep-freeze (-70°C). Protect from light. Hygroscopic. This product is stable for 3 years as supplied.

Toxicity: MSDS available upon request.

Reference: Mata, J.E., et al. 1997. *Toxicol. Appl. Pharmacol.* **144,** 189.

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Revised: 14-November-00

CALBIOCHEM®

PIPER

Cat. No.	Size
528120	10 mg

Synonyms: N,N′-*bis*[2-(1-Piperidino)ethyl]-3,4,9,10-perylenetetracarboxylic Diimide; Telomerase Inhibitor IV

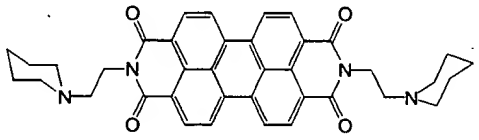
Description: A perylene-based ligand that potently inhibits human telomerase by binding to G-quadruplex DNA. The strongest binding site for PIPER appears to be the 3′-boundary of the G-quadruplex. Can also bind non-specifically to nucleic acids. May be useful as an antiproliferative agent.

Form: Dark purple solid. Packaged under an inert gas.

Molecular Weight: 614.7

Molecular Formula: $C_{38}H_{38}N_4O_4$

Structure:



Purity: ≥95% by HPLC

Solubility: 5% Acetic acid (50 mg/ml)

Storage: Freezer (-20°C). Protect from light. This product is stable for 3 years as supplied.

Toxicity: MSDS available upon request.

Reference: Fedoroff, O.Y., et al. 1998. *Biochemistry* **37,** 12367.

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CALBIOCHEM®

Telomerase Inhibitor V

Cat. No. 581005

Size

10 mg

Synonym: 2,6-*bis*[3-(N-Piperidino)propionamido]anthracene-9,10-dione

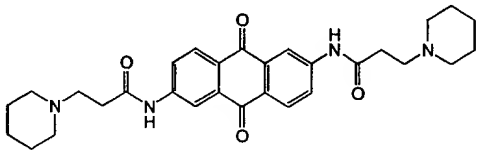
Description: A non-nucleoside 2,6-diaminoanthraquinone derivative that intercalates DNA and forms a discrete binary complex with G-quadruplex structures, resulting in telomerase inhibition (IC_{50} = 4.5 µM). Does not inhibit Taq polymerase even at concentrations of 50 µM. A potential anticancer agent that does not exhibit significant mutagenic activity.

Form: Yellow-brown solid. Packaged under an inert gas.

Molecular Weight: 516.6

Molecular Formula: $C_{30}H_{36}N_4O_4$

Structure:



Purity: ≥85% by HPLC

Solubility: 2% Acetic acid (10 mg/ml)

Storage: Freezer (-20°C). Protect from light. Hygroscopic. This product is stable for 2 years as supplied.

Toxicity: MSDS available upon request.

References:

Perry, P.J., et al. 1998. *J. Med. Chem.* **41,** 3253.
Venitt, S., et al. 1998. *J. Med. Chem.* **41,** 3748.
Agbandje, M., et al. 1992. *J. Med. Chem.* **35,** 1418.

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CALBIOCHEM®

Telomerase Inhibitor VI, Sodium Salt

Cat. No. 581006

Size 100 nmol

Synonyms: 2'-*O*-MeRNA; 5'-2'OMe[C(ps)A(ps)GUUAGGGUU(ps)A(ps)G]-3'

Description: A 13-nucleotide 2'-*O*-MeRNA possessing terminal phosphorothioate linkages. Potently inhibits telomerase activity (IC_{50} = 2 nM at 23°C and 3 nM at 37°C).

Form: Lyophilized solid. Packaged under an inert gas.

Molecular Weight: 4691.8

Sequence: 5'-2'OMe[C(ps)A(ps)GUUAGGGUU(ps)A(ps)G]-3'

Purity: ≥ 90% by PAGE and HPLC

Solubility: H_2O

Storage: Deep freeze (-70°C). Hygroscopic. Protect from light. This product is stable for 2 years as supplied.

Toxicity: MSDS available upon request.

References:

Herbert, B.-S., et al. 1999. *Proc. Natl. Acad. Sci. USA* **96,** 14276.

Pitts, A.E., and Corey, D.R. 1998. *Proc. Natl. Acad. Sci. USA* **95,** 11549.

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CALBIOCHEM®

Telomerase Inhibitor VII, Sodium Salt

Cat. No. 581009

Size: 75 nmol

Synonym: TAG9-2~; 5'-d(GGG~GGG)-3'

Description: A hexameric phosphorothioate oligonucleotide (PS-ODN) TAG9-2~ (wherein a pair of three-base oligomers are separated by a nine carbon spacer) that inhibits telomerase activity *in vitro* (Minimum inhibitory concentration = 1- 3 µM). *Note:* 75 nmol = 174 µg.

Form: Lyophilized solid. Packaged under an inert gas.

Molecular Weight: 2321.5

Purity: ≥90% by PAGE

Solubility: H_2O

Storage: Deep-freeze (-70°C). Protect from light. Hygroscopic. Following reconstitution, aliquot and freeze (-70°C). This product is stable for 3 years as supplied. Stock solutions are stable for 6 months at -70°C.

Toxicity: MSDS available upon request.

Reference: Page, T.J., et al. 1999. *Exp. Cell Res.* **252,** 41.

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See Reference Attached

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The Cytotoxic Effects of Single-Stranded Telomere Mimics on OMA-BL1 Cells

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James R. Neff,¶ and Patrick L. Iversen*†‡,1

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Telomerase is a ribonucleoprotein that adds 5′-d(TTAGGG)-3′ hexameric repeats onto the 3′ ends of chromosomes. High telomerase activity has been associated with immortal cells, transformed cells, mitogenic stimulation, and proliferative diseases. It is not clear what phenotype would be observed by transient inhibition of telomerase. Studies were designed to inhibit telomerase activity using a series of S-ODN telomere sequence motifs. The studies evaluated the length, hydrogen bonding, and sequence requirements of telomerase inhibition using the TRAP assay and a bioassay measuring cell viability following exposure to the compounds. In addition, we have also studied the role of the 3′ end and secondary structure of telomere mimics on telomerase inhibition. Observations reveal that sensitivity to the S-ODNs may not require hybridization to an antisense target but required guanine nucleotides on the 3′ end for cells in culture and telomerase inhibition *in vitro*. The importance of H bonding and the requirement for a free 3′ end for the activity of those compounds has also been demonstrated. However, transient inhibition of telomerase is not cytotoxic to all immortal cells and is not sufficient to explain the mechanism of cytotoxicity of these short oligonucleotides. © 1999 Academic Press

Key Words: **phosphorothioate oligonucleotide; telomere; telomerase inhibitor; lymphoma; sarcoma; drug development.**

INTRODUCTION

The ends of chromosomes, known as telomeres, are characterized by tandem hexameric repeats of the sequence, 5′-d(TTAGGG)-3′. The telomerase enzyme is a ribonucleoprotein that can add these hexameric repeats onto the 3′ ends of telomeres. The telomere has been proposed to act as a "mitotic clock," shortening with each round of cell division until it reaches a minimum critical length causing the cellular senescence [1]. Up-regulation of telomerase activity reverses the telomere-shortening phenomenon resulting in an extended mitotic clock and is important in maintaining an immortal phenotype [2]. Telomerase activity has been detected in 98% of immortal cell lines and 95% of biopsy tumor tissue [3]. In contrast to tumor cells which express high levels of telomerase, normal adult somatic tissue has very low, if any, telomerase activity [4, 5]. The telomerase enzyme is induced in cells following exposure to phorbol esters, UV light, and HPV [6–8]. The retroviral-mediated expression of hTERT, the catalytic subunit of human telomerase, in normal human fibroblasts resulted in elongated telomeres and extended life span [9, 10]. The results of these experiments, taken as a whole, suggest a role for telomerase in the transformation process and that transient inhibition of telomerase might cause a change in the phenotype of the cell or cell death.

Telomerase inhibition as a possible therapeutic modality is being pursued through a variety of technologies including ribozymes, antisense, and traditional drug and natural product screening [11–14]. However, the telomerase knockout mouse was surprising in its lack of phenotype in early generations [15]. Therefore, these studies were designed to investigate the strong correlation between telomerase inhibition and observable effect.

We have shown previously using the telomere repeat amplification protocol (TRAP) assay that a short phosphorothioate oligodeoxynucleotide (S-ODN) telomere sequence motif composed of the sequence, 5′-d(TTAGGG)-3′, referred to as TAG-6 can inhibit telomerase activity *in vitro* [16]. This same compound was found to have antiproliferative effects *in vitro* and *in vivo* against a Burkitt's lymphoma cell line and xenographs in nu/nu C57 Black mice. These observations have led us to design the current studies to define some of the elements that are important for inhibition of

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telomerase and decreased cell viability in immortal telomerase positive cells with S-ODN telomere mimics.

MATERIALS AND METHODS

Cell culture. OMA-BL1 cells are derived from a Burkitt's lymphoma and were established and characterized by Joshi *et al.* [17]. Cell lines and primary cultures were maintained in RPMI 1640 medium (Sigma, St. Louis, MO), supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY), penicillin G (50 units/ml), and streptomycin (50 μg/ml) (Sigma). The cells were subcultured at 3- to 4-day intervals to a density of 1×10^6 cells/ml in a 75-cm^2 flask containing 30 ml of medium and incubated at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Primary cultures were maintained for one to two passages before viability was determined.

Oligonucleotide synthesis and purification. The ODNs were synthesized on a 1-μmol scale with an Applied Biosystems Model 391 DNA synthesizer using the recommended protocols for synthesis of PS ODNs. The 3′ glycerol S-ODN was synthesized using a commercially available CPG column with the glycerol molecule preattached (Glen Research, Sterling, WV). The 18-mer-containing 7-deaza-2′-deoxyguanosine at positions 5 and 17, 5′-d(TTAGGGTTAGGGTTAGGG)-3′ (bolded G's represent the 7 deaza sites) was synthesized using the 7-deaza-dG-CE phosphoramidite (Glen Research). The Spacer phosphoramidite 9 (Glen Research) was used to produce the 5′-d(GGG~GGG)-3′ (TAG 9-2~) and 5′-d(TTA~TTA)-3′ (TAG 9-1~) molecules. S-ODNs containing abasic sites were synthesized using the dSpacer Phosphoramidite (Glen Research). The glyoxalated TAG 9-2, 5′-d(GGGTTAGGG)-3′, and TAG 9-1, 5′-(TTAGGGTTA)-3′, were glyoxalated according to the method of Birnboim and Mitchel [18]. Glyoxalation was verified using spectroflouroscopy [19]. The random S-ODNs were synthesized by mixing equimolar aliquots of all four nucleotides together and using that bottle as the amidite mix for the base that we chose to randomize.

Cell viability assays. The cell viability assay was modified from a method by Watanabe *et al.* based on the reduction of MTT (3,4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide, Sigma) by mitochondrial dehydrogenase in viable cells to a formazan product that can be measured spectrophotometrically [20]. Viability assays were carried out in 96-well plates with the addition of 100 μl of medium containing 3000 cells into each well. Ten microliters of medium containing ODNs were added to the wells for final concentrations of 0.3, 1, 3, and 10 μM. The plates were incubated for 72 h and then lysed using 0.04 N HCl in isopropanol. The absorbance was measured at 540 nm on a 96-well microplate reader. The EC_{50} for each compound was determined using Graphpad/Prism software.

Randomization of single bases in S-ODNs. Bases were randomized by combining the phosphoramidites in equimolar amounts of all four nucleotides into a single bottle and synthesizing the S-ODNs with one randomized base by drawing from that bottle position on the synthesizer. Data are expressed as the percentage of viable cells compared to untreated cells. The percentage of viable cells was calculated by dividing the average absorbance at 540 nm (A^{540}) of treated cells with the A^{540} of untreated cells in each viability assay. Cells were seeded in 96-well plates at a density of 3000 cells/100 μl of medium. ODNs were added at a concentration of 10 μM. Cultures were incubated at 37°C for 72 h. MTT assays were then performed as described on the cultures in triplicate and these data were analyzed using Graphpad/Prism software.

TRAP assay. The TRAP assays were performed using a telomerase detection kit (Oncor, Gaithersburg, MD). Cell lysates were prepared from OMA-BL-1 cells according to the recommended procedure and were flash frozen in liquid nitrogen and stored at −70°C until use. Two microliters of lysates was used for each TRAP reaction containing 3 ng protein/μl along with 3 μl of S-ODN or control solution. Forty-five microliters of the TRAP assay PCR mix, containing ^{32}P-labeled TS primer, was then added to each tube. The tubes were incubated for 30 min at 30°C. Two-step PCR was then performed on the tubes (94°C/30 s, 60°C/30 s for 30 cycles). The PCR products were then run on a 12.5% nondenaturing PAGE in TBE buffer. The gels were run for 3 h at 300 V. The gels were then fixed in 0.5 M NaCl, 50% ethanol, 40 mM sodium acetate (pH 5.5), for 2 min and then exposed without drying to storage phosphor screen and visualized on a PhosphorImager using ImageQuant analysis software (Molecular Dynamics, Sunnyvale, CA).

TABLE 1

Effect of Length of Telomere Mimic on Cytotoxicity in OMA-BL1 Cells in Culture

S-ODN sequence (5′–3′)	EC_{50} (μM)
G[a]	13.36 ± 2.16
GG	12.14 ± 2.00
GGG	15.59 ± 3.12
GGGG	2.83 ± 0.18
TTAG	>20
TTAGG	>20
TTAGGG	2.00 ± 0.20
TTAGGGT	>20
TTAGGGTT	>20
TTAGGGTTA	12.13 ± 1.61
GGGTTAGGG	2.94 ± 0.22
TTAGGGTTAG	6.54 ± 3.96
TTAGGGTTAGG	13.21 ± 1.69

[a] Phosphorothioate guanosine triphosphate deoxyribonucleic ac

Polymerase chain reaction. PCR reactions were performed wi 1.25 units of *Taq* polymerase (Promega) according to manufacture protocols to amplify the pUC19 plasmid using Lac+/− primers. Tl indicated amount of oligonucleotide was also added to each reactio The reaction was heated to 98°C for 2 min. 25 PCR cycles were the performed (1 min at 94°C/30 s at 42°C/2 min at 72°C). The PC products were visualized under UV light following electrophoresis o a 1% agarose, 1× TBE gel containing ethidium bromide.

Statistical analysis. Statistical analysis was done by performin one-way analysis of variance and Tukey's multiple comparison tes The EC_{50} for each compound was determined by nonlinear regres sion. All analysis was done using GraphPad/Prism software v.2. 1995.

RESULTS

Length of telomere mimics effect cytotoxicity in OMA-BL1 cells. To study the length requirements of the telomere mimic sequences we tested a matrix of compounds with length 1–24 bases which contain the telomere sequence repeat motif. PS-ODN concentrations above 10 μM inhibit cell growth in a non-sequence-specific manner. Therefore, we have considered oligomers which have EC_{50} values above 10 μM to be noncytotoxic relative to those oligomers with sequence-specific toxicity. The longer TAG sequences tended to show lower EC_{50} values than the shorter compounds (Table 1). We also observed that the exceptionally effective smaller oligonucleotides all contain three to four guanines at the 3′ end with the exception of the

TABLE 2

Comparison of Telomerase Inhibition and Cytotoxicity (MTT Assay)

S-ODN sequence (5′–3′)	Cytotoxicity EC_{50} (μM) $n = 4$	(MIC) telomerase inhibition (μM)
GGGTTAGGG	2.93 ± 0.22	0.3
GGTTAGGGT	13.63 ± 2.97	0.3
GTTAGGGTT	3.67 ± 0.32	3–10
TTAGGGTTA	12.13 ± 1.61	3–10
TAGGGTTAG	12.70 ± 1.63	1–3
AGGGTTAGG	4.36 ± 0.60	3–10
GGG~GGG (TAG 9-2~)	2.13 ± 0.13	1–3
GGGĜGG (TAG 9-2^)	1.67 ± 0.14	1–3

base S-ODN which contained two guanines at the 3′ end. Further, we observed an inconsistent dose–response curve with the 18- and 24-base sequences [5′-d(TTAGGGTTAGGGTTAGG)-3′ and 5′-d(TTAGGGTTAGGGTTAGGGTTAGGG)-3′, respectively], possibly due to formation of secondary structures. We found that there was no advantage to the longer molecules and we considered the exclusion of possible interactions with mRNA through hydrogen bonding important in establishing mechanism of action. Therefore, we chose to study 9-base telomere mimics which allow us to vary the sequence and retain one complete telomere motif but which would not be expected to form stable duplexes with complementary sequences.

The frame of the telomere mimic within the S-ODN affects cytotoxicity and TRAP inhibition. Based on the results of Table 1 a series of S-ODNs were synthesized in which the telomere motif is shifted 1 base for each member and again tested for antiproliferative effects with OMA-BL1 cells and for their ability to inhibit telomerase *in vitro* (Table 2). These experiments showed that the 5′-d(GGGTTAGGG)-3′ (TAG 9-2) exhibited significantly greater efficacy than the other 9 mers. There is no clear pattern to the preference for certain sequences though the cytotoxic response was clearly dependent on which frame the telomeric sequences were presented in. The results of the telomerase assay were interesting in that there was no correlation between those frames which inhibited the TRAP assay and cytotoxicity with the exception of TAG 9-2 which was cytotoxic and inhibited TRAP and 5′-d(TTAGGGTTA)-3′ (TAG 9-1) which was not cytotoxic and did not inhibit TRAP. Further studies employ the TAG 9-1 as an inactive control since it appears to have no significant effect on the growth of OMA-BL1 cells in culture.

Role of selected bases and position in sequence. To determine the importance of each base in the TAG 9-2 S-ODN, six S-ODNs were synthesized, each having one base randomized. Only the first six bases were randomized because the importance of the guanine motif at the 3′ end had already been established by previous experiments. When OMA-BL1 cells were treated with these compounds, a clear pattern emerged (Fig. 1). When bases 1, 2, or 3 were randomized the S-ODNs were inactive while randomization of bases 4, 5, or 6 produced S-ODNs which remained cytotoxic.

Effects of spacer molecules in S-ODNs. Based on the results of the previous experiment it was hypothesized that bases 4, 5, and 6, the TTA portion of TAG 9-2, could be replaced with a spacer molecule, without decreasing its efficacy. The TTA portion of TAG 9-2 and the GGG centered in TAG 9-1 were replaced with a commercially available nine-carbon spacer molecule denoted with an approximation symbol. The S-ODNs contain a pair of three-base oligomers separated by the nine-carbon spacer and were synthesized with the sequences 5′-d(GGG~GGG)-3′ (TAG 9-2~) and 5′-d(TTA.~TTA)-3′ (TAG 9-1~). When OMA-BL-1 cells were treated with TAG 9-2~, a decrease in the cell viability was observed when compared with the normal TAG 9-2 S-ODN (Table 2). The TAG 9-1 spacer S-ODN did not show any significant effects on the viability of OMA-BL-1 cells (data not shown).

In addition to the nine-carbon spacer an abasic site spacer was also used to replace the 5′-TTA-3′ portion of TAG 9-2. The abasic site is a deoxyribose sugar without a base, with a sulfur atom replacing one of the nonbridging oxygen atoms in the phosphodiester bond, making it a phosphorothioate. We denote the presence of this spacer with a caret. This spacer was used to determine whether or not the increased efficacy seen with the TAG 9-2~ S-ODN was due to an intrinsic property of the nine-carbon spacer or if it was merely

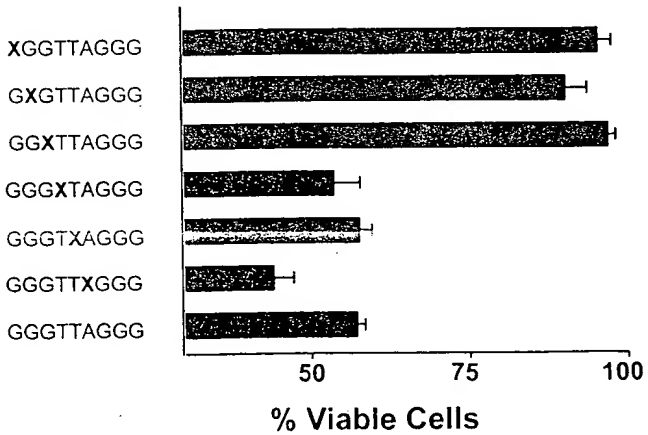

FIG. 1. Effect of replacing specific bases with random bases on the cytotoxicity of TAG 9-2 on OMA-BL1 cells. The x-axis is cell viability as measured by the MTT assay; the y-axis is the modification of each oligomer with X denoting the randomized base. Cells were seeded in 96-well plates at a density of 3000 cells/100 μl of medium and incubated at 37°C for 72 h; $n = 4$ for all concentrations tested.

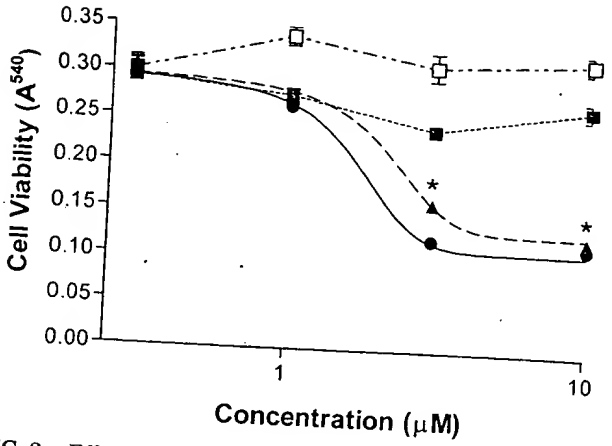

FIG. 2. Effect of replacing bases 4, 5, and 6, 5′d(TTA)-3′, with an abasic site spacer molecule (^) or with a spacer molecule (~) on the cytotoxicity of TAG 9-2 on OMA-BL1 cells. The y-axis is cell viability as measured by the MTT assay; the x-axis is the dose of S-ODN. Cells were seeded in 96-well plates at a density of 3000 cells/100 μl of medium and incubated at 37°C for 72 h; $n = 4$ for all concentrations tested. (■) Untreated cells, (●) cells treated with TAG 9-2^, (▲) cells treated with TAG 9-2~, and (□) cells treated with TAG 9-1~. *Values which are significantly different from untreated and control cells ($P < 0.01$).

the result of the substitution of any generic spacer. When OMA-BL-1 cells were treated with TAG 9-2^ and TAG 9-2~ it was observed that TAG 9-2^ was essentially equivalent to TAG 9-2~ (Fig. 2). In parallel with this experiment TAG 9-1^ with the three guanine nucleotides in the middle of the molecule replaced by the abasic site spacer was also tested on OMA-BL-1 cells and was found to have no cytotoxicity (data not shown).

Cytotoxicity of TAG 9-2 in cell lines and primary tumor cells in culture. In order to evaluate whether the cytotoxicity of the TAG 9-2 compound was specific to OMA-BL1 cell line, a variety of transformed cells and cells derived from primary cultures were challenged with TAG 9-2 to determine cytotoxicity (Table 3). Sensitivity between cell lines varied even among other Burkitt's lymphoma-derived cell lines such as the Jurkat and Raji lines which are not sensitive to the TAG compounds. SK-EW and the OH-931 cell lines exhibited a very strong response to the compound. The primary tumor-derived cells were very sensitive to the TAG 9-2 compound with the exception of dermatofibrosarcoma- and a esthesioneuroma-derived culture.

The role of hydrogen bonding in cytotoxicity. We evaluated the possibility that the mechanism which produced cytotoxicity involved base pairing or hydrogen bonding. These studies involved blocking the hydrogen-bonding capability of the TAG 9-2 S-ODN by glyoxalation. The glyoxalation reaction produces an etheno- group on guanine nucleotides that effectively blocks hydrogen bonding [18]. The yield of the reaction is not 100%, but the etheno- group has distinct excitation and emission spectra that allowed us to verify the presence. When OMA-BL1 cells were treated with the glyoxalated TAG 9-2 compound, a significant decrease in the efficacy of the compound was observed, most notably at the 10 μM concentration (Fig. 3). Cells treated with TAG 9-1 and glyoxalated TAG 9-1 showed no cytotoxicity (data not shown). The lack of effect for glyoxalated TAG 9-1 indicates that the glyoxalation itself does not result in a toxic product.

Role of a free 3′ end in the cytotoxic effects. Since telomerase is known to add repeats onto the 3′ ends of chromosomes it was hypothesized that if TAG 9-2 was acting as a substrate for the telomerase enzyme, sterically blocking the 3′ end of the molecule might decrease its efficacy. To test this hypothesis TAG 9-2 was synthesized with a glycerol molecule covalently linked to the 3′ hydroxyl group. The viability of OMA-BL-1 cells treated with the TAG 9-2 glycerol compound was increased when compared with the normal TAG 9-2 S-ODN; again, this effect was most notable at higher concentrations (Fig. 4). No effect was observed on cells treated with TAG 9-1 or TAG 9-1 with an equivalent 3′ glycerol.

Loss of efficacy in longer telomere mimics due to secondary structures. Guanine motifs in nucleic acids can form secondary structures such as g-quartet and g-wire, in addition to single-stranded structures. These secondary structures can be the result of intra- and interstrand associations. The possibility exists that the longer TAG sequences can form these associations more readily and that these associations might alter

TABLE 3

Viability of Cell Lines and Cultured Primary Tumor Cells in the Presence of TAG 9-2

Cell line	EC_{50} (μM) ($n = 2$)
SJR H-30	>10
SK-EW	0.03
OH 931	<0.01
Jurkat	>10
Raji	>10

Sample	Diagnosis	EC_{50} (μM) ($n = 2$)
1	Chordoma	<0.01
2	Osteosarcoma	<0.01
3	Esthesioneuroma	7.9
4	MFH	0.19
5	Ewings Sarcoma	0.03
6	Dermatofibrosarcoma	>10
7	Liposarcoma	<0.01
8	Osteosarcoma	0.01
9	Osteosarcoma	<0.01
10	Osteosarcoma	<0.01
11	Ewings Sarcoma	0.37
12	Chondrosarcoma	0.43

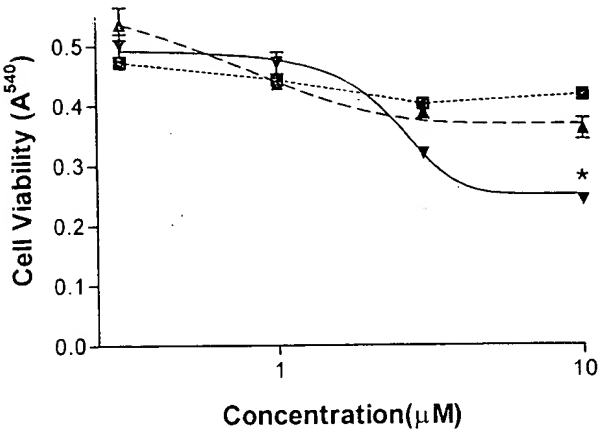

FIG. 3. Effect of partially blocking hydrogen bonding on the cytotoxicity of TAG 9-2 on OMA-BL1 cells. The y-axis is cell viability as measured by the MTT assay; the x-axis is the dose of S-ODN. Cells were seeded in 96-well plates at a density of 3000 cells/100 μl of medium and incubated at 37°C for 72 h; $n = 3$ for all concentrations tested. (■) Untreated cells, (▲) cells treated with TAG 9-2 glyoxalated, and (▼) cells treated with TAG 9-2. *Values which are significantly different from untreated and glyoxalated TAG 9-2-treated cells ($P < 0.05$).

the way these TAG compounds interact with the telomerase enzyme [21]. To test this hypothesis two guanine nucleotides that contain a deaza group at the G-7 position were incorporated into the TAG 18 S-ODN at position 5 and 17 during synthesis (see Materials and Methods). The location of the deaza group (G-7) sterically blocks the formation of a variety of secondary structures. The TAG 18 S-ODN was used for these experiments because the longer telomere mimics are more likely to form higher order structures. When OMA-BL1 cells were treated with the TAG 18 7-deaza compounds an increased cytotoxic effect was observed than for cells treated with the normal TAG 18 (Fig. 5).

Inhibition of telomerase enzyme activity in vitro with S-ODNs. A series of TRAP assays was performed on modified TAG 9-2 S-ODNs (Fig. 6). S-ODNs can inhibit a number of polymerases in a non-sequence-specific manner at concentrations of 3 to 10 μM so each oligomer was evaluated for inhibition of *Taq* polymerase amplification of plasmid DNA which is shown in the bottom of Fig. 6. The S-ODNs which were capable of hydrogen bonding and which have a 3′ G present were efficient at inhibiting TRAP at 0.1 μM but failed to inhibit *Taq* polymerase at up to 3 μM. The S-ODNs in which the 3′ end was modified with a glycerol or in the glyoxylated TAG 9-2 no longer inhibited TRAP efficiently. TAG 9-1 did not inhibit the TRAP until 10 μM which is the concentration that phosphorothioate oligonucleotides will inhibit the reaction in a non-sequence-specific manner. These data suggest that the ability to present a free 3′ end and the ability to hydrogen bond are important to the mechanism of action of the TAG compounds both as cytotoxic agents and as telomerase inhibitors.

DISCUSSION

Since the discovery that telomerase activity is highly associated with many disease states it has been a goal of researchers to develop molecules that are capable of inhibiting this enzyme [22–27]. Telomerase inhibitors have been proposed for use as anticancer therapeutics [14, 28]. Short oligomers have been shown to be capable of inhibiting telomerase *in vitro* and have been tested *in vivo* for their antineoplastic and antimetastatic properties [12, 16].

The length studies presented demonstrate that although the longer oligomers form more stable hybrid duplexes, their ability to illicit a cytotoxic response is not dependent on the length. Single-stranded telomeric probes of sufficient length can form hairpin duplex, triplex, and quadruple helical structures through G:G pairing for which specific protein complexes are associated [29–35]. The G:G pairing may result in aggregation or DNA:protein complexes which prevent access to the site of action. The fact that the length of the telomere mimic did not confer an advantage over the short oligomers leads to the hypothesis that the cytotoxic effects were due to aptameric effects of the telomeric sequence. The 5′-d(GGGG)-3′ molecule, although not as cytotoxic as some of the telomere mimics, showed considerable effect supporting the hypothesis that the G-motif is an important component to the cytotoxicity of telomere mimics.

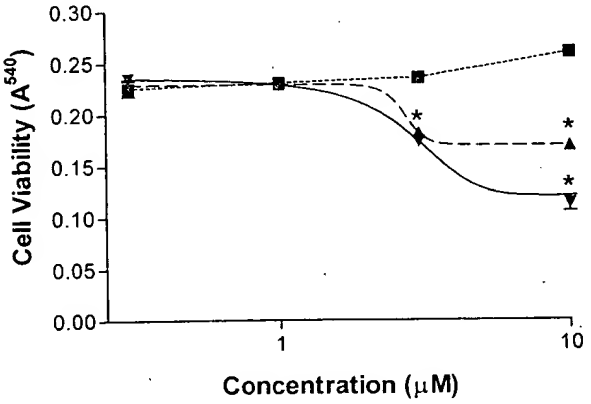

FIG. 4. Effect of sterically blocking the 3′ end of TAG 9-2 with glycerol on its cytotoxicity on OMA-BL1 cells. The y-axis is cell viability as measured by the MTT assay; the x-axis is the dose of S-ODN. Cells were seeded in 96-well plates at a density of 3000 cells/100 μl of medium and incubated at 37°C for 72 h; $n = 3$ for all concentrations tested. (■) Untreated cells, (▲) cells treated with TAG 9-2 3′ glycerol, and (▼) cells treated with TAG 9-2. *Values which are significantly different from untreated cells ($P < 0.05$).

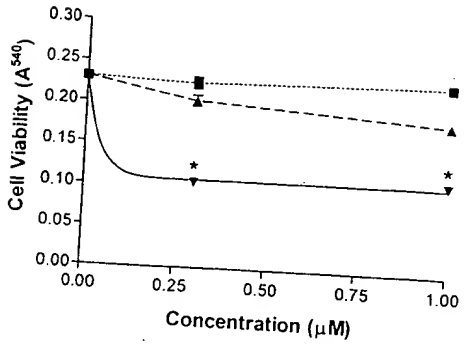

FIG. 5. Effect of blocking secondary structures on TAG 18, 5′-d(TTAGGGTTAGGGTTAGGG)-3′, on its cytotoxicity on OMA-BL1 cells. The *y*-axis is cell viability as measured by the MTT assay; the *x*-axis is the dose of S-ODN. Cells were seeded in 96-well plates at a density of 3000 cells/100 μl of medium and incubated at 37°C for 72 h; $n = 3$ for all concentrations tested. (■) untreated cells, (▲) cells treated with TAG 18, and (▼) cells treated with TAG 18 containing a 7-deaza-guanine at the 5 and 17 positions. *Values which are significantly different from untreated and TAG 18-treated cells ($P < 0.05$).

The studies in which six telomere mimics of nine-base length were synthesized and the sequence was shifted one base for each were designed to test whether the requirements for TRAP inhibition could be separated from the cytotoxic effects that were seen with these compounds. These studies demonstrate that while both cytotoxic effects and inhibition of TRAP required sequences in a particular frame, the frame requirements were not the same for each endpoint. In fact, small changes in the six 9-mer S-ODNs creates a matrix of oligomers capable of cytotoxic responses an TRAP inhibition, no response in either, cytotoxici with little TRAP inhibition, and no cytotoxicity wi significant inhibition of TRAP. These observation would suggest that inhibition of enzyme activity a measured by TRAP is not sufficient to explain th cytotoxic effects of these compounds.

Randomization of each of the first six bases of th S-ODNs confirmed our earlier observations tha guanosine at both the 3′ and the 5′ ends is important t the observed cytotoxicity. The role of the bases flanked by the poly-G motif is apparently less important than the presentation of the poly-G motif at the ends since replacing these bases with either a nine-carbon chain spacer or an abasic site did not diminish either the cytotoxicity or the telomerase inhibition of the S-ODNs.

Oligonucleotides with the ability to form G-tetrads have been shown to inhibit telomerase and produce aptameric effects *in vitro* and *in vivo* [21, 36, 37]. Synthesis of an 18-base telomere mimic which incorporated 7-deaza-G nucleotides was designed to limit the S-ODNs ability to form a dimer or quartet structure by preventing Hoogstein G:G pairing. This modification increased the cytotoxicity of the compound in OMA-BL1 cells suggesting that there are other considerations than the ability to form a quartet structure. It is possible that the longer 7-deaza S-ODN is less likely to aggregate and may have preferential uptake by the cells in culture.

Studies in which short telomere mimics were ad in-

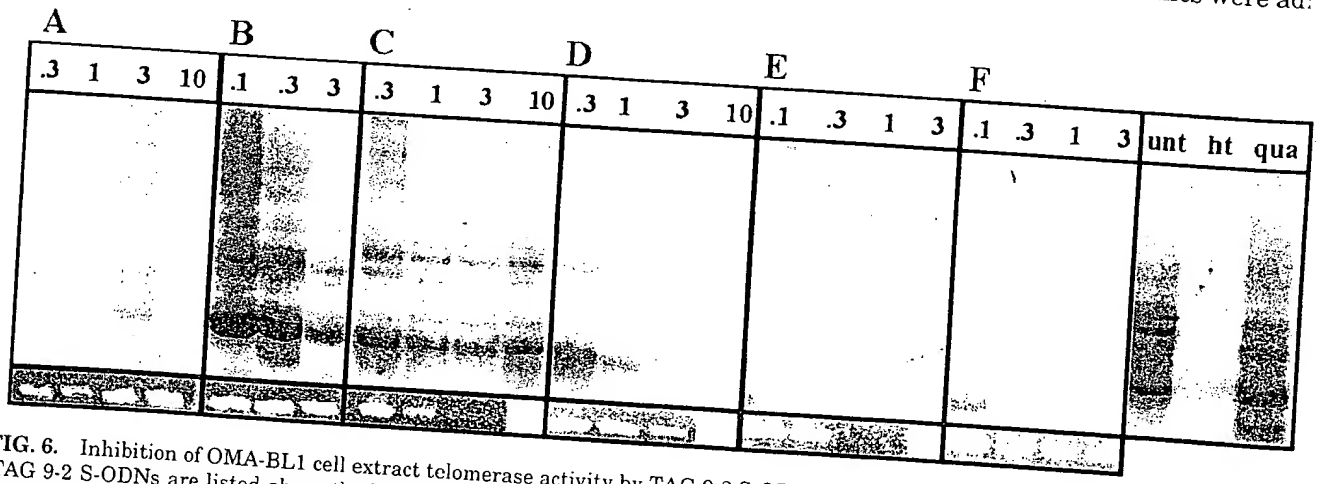

FIG. 6. Inhibition of OMA-BL1 cell extract telomerase activity by TAG 9-2 S-ODN *in vitro* as measured by TRAP assay. Concentrations of TAG 9-2 S-ODNs are listed above the lanes in micromolar amounts. Segment A is cell extracts with TAG 9-2 added. Segment B is cell extracts with TAG 9-1 added. Segment C is cell extracts with glyoxalated TAG 9-2 added. Segment D is cell extract with TAG 9-2 with a glycerol molecule attached at the 3′ end. Segment E is cell extract with TAG 9-2 with an abasic site molecule replacing bases 4, 5, and 6. Segment F is cell extract with TAG 9-2 with the nine-spacer molecule replacing bases 4, 5, and 6. The unt lane is cell extracts without treatment by any S-ODN. The ht lane is heat-treated cell extracts used as a control to ensure that telomerase is responsible for the elongation. The qua lane is a control lane used to control for the efficiency of the assay. It consists of the telomere ladder that is added to the TRAP reaction without lysates. If the PCR portion of the assay works properly a 6-bp ladder will be present in this lane. Ethidium bromide-stained bands from PCR products in the presence of the indicated concentrations of oligomer are presented at the bottom of each lane.

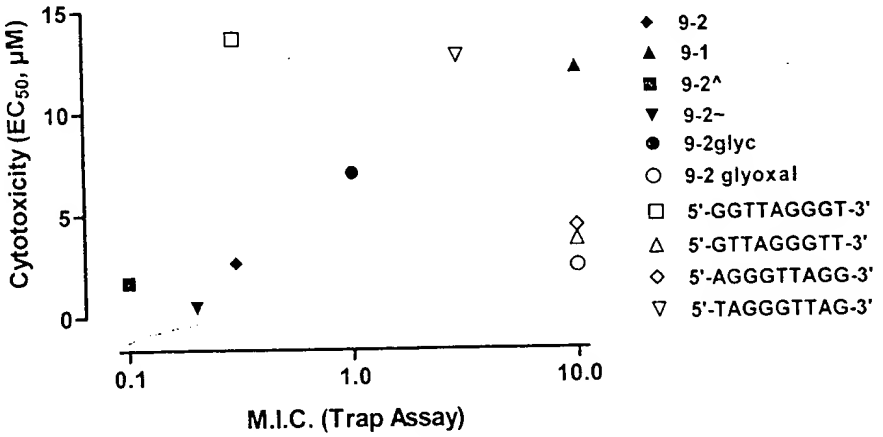

FIG. 7. The relationship between cytotoxicity on OMA-BL1 cells and inhibition of telomerase in the TRAP assay. On the x-axis is the minimum inhibitory concentration for the TRAP assay as estimated from the TRAP assays in Fig. 6. The y-axis is the log EC_{50} of the cytotoxicity assays. The EC_{50} was generated using Graphpad/Prism software.

istered to nude mice bearing a human Burkitt's lymphoma tumor revealed that the S-ODNs were excreted in urine as six-base DNA-conjugated ladders, hence the hypothesis that the oligonucleotide telomere mimics act as competitive substrates in the telomerase complex. Studies presented here modified the 3′ end by conjugating it with glycerol which would prevent enzymatic telomere additions to the oligonucleotide. The 3′ glycerol modification ablated both cytotoxicity and inhibition of telomerase consistent with our hypothesis.

Several strategies were employed to block sites on the TAG 9-2 molecule to better understand which contact points are important to the cytotoxic effects. The decrease in efficacy by treating the S-ODN with glyoxal suggests that H bonding is one component of the cytotoxic mechanism. Since the glyoxylation reaction does not produce a completely glyoxylated product it is likely that inhibition of the cytotoxic response was not complete due to unglyoxylated S-ODN remaining in the product. These data do, however, support the hypothesis that the mechanism of action involves hydrogen bonding with the bases. The blocking of the 3′ end of the molecule with glycerol reduces the toxicity suggesting that the free 3′ end is an important component to the response in OMA-BL1 cells.

Cytotoxicity varies among several cell types tested with OMA-BL1 cells exhibiting a sensitivity not found in two other Burkitt's lymphoma-derived cell lines. This is not surprising since cellular interactions with oligonucleotides have been shown to be cell type specific [38]. The sequence-specific cytotoxic effects of the telomere mimics may be due to enhanced uptake by cells or cellular compartments and/or binding to receptors or proteins which lead to cell death. The data generated with the established cell lines and primary tumors suggest that while sensitivity varies, many cell types are sensitive to these short oligomers.

The ability of TRAP inhibition to predict cytotoxicity in OMA-BL1 cells correlates well for the modified TAG 9-2 S-ODNs; however, the other S-ODN data clearly demonstrate that inhibition of TRAP cannot account for the cytotoxicity seen in many of the telomere mimics (Fig. 7). Our previous studies have demonstrated that short telomere mimics are capable of producing apoptosis [16]. Hence, the fact these oligomers mimic the telomere may be more significant than their ability to inhibit telomerase.

These observations cause us to propose a functional endogenous role for telomere fragments produced in cells. Such fragments may arise as the result of oxidative stress which generates superoxide anion and hydroxyl radical [39]. The hydroxyl radical preferentially generates 8-oxo-G adducts which tends to hydrolyze resulting in DNA strand breaks [39–42]. Since telomeres contain significant single-stranded DNA composed of 50% guanine it would be anticipated to generate short oligomer fragments following oxidative stress. Further, the resulting sequence motif would generate sequences of G_nTTAG_n where n = 1–3. These sequences would be five to nine bases in length or longer if the oxidative insult is smaller. We observe the expected sequence motif to be most effective in causing cell death in OMA-BL-1 cells and inhibiting TRAP.

Current cancer chemotherapy involves inducing cell death through the generation of hydroxyl radicals [40]. Telomeric DNA is more sensitive to the damage induced by selected chemotherapy than chromosomal DNA [44]. The cell death observed by the short oligomers described here may represent mimics of endogenous cell signaling downstream from the oxidative stress. The oligomer telomere mimic may represent a novel approach to cancer chemotherapy.

REFERENCES

1. Lundblad, V., and Szostak, J. W. (1989). A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell* **57,** 633–643.
2. Morin, G. B. (1989). The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* **59,** 521–529.
3. Kim, N. W., Piatyszek, M. A., Prowse, K. R., Harley, C. B., West, M. D., Ho, P. L., Coviello, G. M., Wright, W. E., Weinrich, S. L., and Shay, J. W. (1994). Specific association of human telomerase activity with immortal cells and cancer [see comments]. *Science* **266,** 2011–2015.
4. Blasco, M. A., Rizen, M., Greider, C. W., and Hanahan, D. (1996). Differential regulation of telomerase activity and telomerase RNA during multi-stage tumorigenesis. *Nature Genet.* **12,** 200–204.
5. Counter, C. M., Hirte, H. W., Bacchetti, S., and Harley, C. B. (1994). Telomerase activity in human ovarian carcinoma [see comments]. *Proc. Natl. Acad. Sci. USA* **91,** 2900–2904.
6. Hiyama, K., Hirai, Y., Kyoizumi, S., Akiyama, M., Hiyama, E., Piatyszek, M. A., Shay, J. W., Ishioka, S., and Yamakido, M. (1995). Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J. Immunol.* **155,** 3711–3715.
7. Ueda, M., Ouhtit, A., Bito, T., Nakazawa, K., Lubbe, J., Ichihashi, M., Yamasaki, H., and Nakazawa, H. (1997). Evidence for UV-associated activation of telomerase in human skin. *Cancer Res.* **57,** 370–374.
8. Klingelhutz, A. J., Foster, S. A., and McDougall, J. K. (1996). Telomerase activation by the E6 gene product of human papillomavirus type 16. *Nature* **380,** 79–82.
9. Bodnar, A. G., Ouellette, M., Frolkis, M., Holt, S. E., Chiu, C. P., Morin, G. B., Harley, C. B., Shay, J. W., Lichtsteiner, S., and Wright, W. E. (1998). Extension of life-span by introduction of telomerase into normal human cells [see comments]. *Science* **279,** 349–352.
10. Vaziri, H., and Benchimol, S. (1998). Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. *Curr. Biol.* **8,** 279–282.
11. Kanazawa, Y., Ohkawa, K., Ueda, K., Mita, E., Takehara, T., Sasaki, Y., Kasahara, A., and Hayashi, N. (1996). Hammerhead ribozyme-mediated inhibition of telomerase activity in extracts of human hepatocellular carcinoma cells. *Biochem. Biophys. Res. Commun.* **225,** 570–576.
12. Norton, J. C., Piatyszek, M. A., Wright, W. E., Shay, J. W., and Corey, D. R. (1998). Inhibition of human telomerase activity by peptide nucleic acids. *Nature Biotechnol.* **14,** 615–619.
13. Sharma, H. W., Maltese, J. Y., Zhu, X., Kaiser, H. E., and Narayanan, R. (1996). Telomeres, telomerase and cancer: Is the magic bullet real? *Anticancer Res.* **16,** 511–515.
14. Sharma, S., Raymond, E., Soda, H., Sun, D., Hilsenbeck, S. G., Sharma, A., Izbicka, E., Windle, B., and Von Hoff, D. D. (1997). Preclinical and clinical strategies for development of telomerase and telomere inhibitors. *Ann. Oncol.* **8,** 1063–1074.
15. Blasco, M. A., Lee, H. W., Hande, M. P., Samper, E., Lansdorp, P. M., DePinho, R. A., and Greider, C. W. (1997). Telomere shortening and tumor formation by mouse cells lacking telomerase RNA [see comments]. *Cell* **91,** 25–34.
16. Mata, J. E., Joshi, S. S., Palen, B., Pirruccello, S. J., Jackson, J. D., Elias, N., Page, T. J., Medlin, K. L., and Iversen, P. L. (1997). A hexameric phosphorothioate oligonucleotide telomerase inhibitor arrests growth of Burkitt's lymphoma cells in vitro and in vivo. *Toxicol. Appl. Pharmacol.* **144,** 189–197.
17. Joshi, S. S., Deboer, J. M., Standjord, S. J., Pirrucello, S. J., Sanger, W. G., Weisenberger, D. D., and Sharp, J. G. (1991) Characterization of newly established human Burkitt's lymphoma cell line, OMA-BL1. *Int. J. Cancer* **47,** 643–648.
18. Birnboim, H. C., and Mitchel, R. E. J. (1978). Prevention of G:C pairing in mouse DNA by complete blocking of guanine residues with glyoxal. *Biochim. Biophys. Acta* **517,** 296–307.
19. Broude, N. E., and Budowsky, E. I. (1971). The reaction of glyoxal nucleic acid components. III. Kinetics of the reaction with monomers. *Biochim. Biophys. Acta* **254,** 380–388.
20. Watanabe, W., Konno, K., Ijichi, K., Inoue, H., Yokota, T., and Shigeta, S. (1994). MTT colorimetric assay system for the screening of anti-orthomyxo- and anti-paramyxoviral agents. *J. Virol. Methods* **48,** 257–265.
21. Zahler, A. M., Williamson, J. R., Cech, T. R., and Prescott, D. M. (1991). Inhibition of telomerase by G-quartet DNA structures. *Nature* **350,** 718–720.
22. Parkinson, E. K. (1996). Do telomerase antagonists represent a novel anti-cancer strategy? [Editorial]. *Br. J. Cancer* **73,** 1–4.
23. Burger, A. M., Double, J. A., and Newell, D. R. (1997). Inhibition of telomerase activity by cisplatin in human testicular cancer cells. *Eur. J. Cancer* **33,** 638–644.
24. Fletcher, T. M., Salazar, M., and Chen, S. F. (1996). Human telomerase inhibition by 7-deaza-2′-deoxypurine nucleoside triphosphates. *Biochemistry* **35,** 15611–15617.
25. Ku, W. C., Cheng, A. J., and Wang, T. C. (1997). Inhibition of telomerase activity by PKC inhibitors in human nasopharyngeal cancer cells in culture. *Biochem. Biophys. Res. Commun.* **241,** 730–736.
26. Sun, D., Thompson, B., Cathers, B. E., Salazar, M., Kerwin, S. M., Trent, J. O., Jenkins, T. C., Neidle, S., and Hurley, L. H. (1997). Inhibition of human telomerase by a G-quadruplex-interactive compound. *J. Med. Chem.* **40,** 2113–2116.
27. Melana, S. M., Holland, J. F., and Pogo, B. G. (1998). Inhibition of cell growth and telomerase activity of breast cancer cells in vitro by 3′-azido-3′-deoxythymidine. *Clin. Cancer Res.* **4,** 693–696.
28. Rhyu, M. S. (1995). Telomeres, telomerase, and immortality [see comments]. *J. Natl. Cancer Inst.* **87,** 884–894.
29. Gualberto, A., Patrick, R. M., and Walsh, K. (1992). Nucleic acid specificity of a vertebrate telomere-binding protein: evidence for G:G base pair recognition at the core-binding site. *Genes Dev.* **6,** 815–824.
30. Williamson, J. R., Raghuraman, M. K., and Cech, T. R. (1989). Monovalent cation-induced structure of telomeric DNA: The G-quartet model. *Cell* **59,** 871–880.
31. Fang, G., and Cech, T. R. (1993). Characterization of a G-quartet formation reaction promoted by the beta-subunit of the Oxytricha telomere-binding protein. *Cell* **74,** 875–885.
32. Veselkov, A. G., Malkov, V. A., Frank-Kamenetskii, M. D., and Dobrynin, V. N. (1993). Triplex model of chromosome ends. *Nature* **364,** 496.
33. Walsh, K., and Gualberto, A. (1992). MyoD binds to the guanine tetrad nucleic acid structure. *J. Biol. Chem.* **267,** 13714–13718.
34. Liu, Z., Frantz, J. D., Gilbert, W., and Tye, B. K. (1993). Identification and characterization of a nuclease activity specific for G4 tetastranded DNA. *Proc. Natl. Acad. Sci. USA* **90,** 3157–3161.
35. Henderson, E. R., Hardin, C. C., Walk, S. K., Tinoco, I., Jr., and Blackburn, E. H. (1987). Telomeric DNA oligonucleotides form novel intramolecular structures containing guanine–guanine base pairs. *Cell* **51,** 899–908.

36. Sharma, H. W., Hsiao, R., and Narayanan, R. (1996). Telomerase as a potential molecular target to study G-quartet phosphorothioates. *Antisense Nucleic Acid Drug Dev.* **6,** 3–7.

37. Burgess, T. L., Fisher, E. F., Ross, S. L., Bready, J. V., Qian, Y. X., Bayewitch, L. A., Cohen, A. M., Herrera, C. J., Hu, S. S., and Kramer, T. B. (1995). The antiproliferative activity of c-myb and c-myc antisense oligonucleotides in smooth muscle cells is caused by a nonantisense mechanism. *Proc. Natl. Acad. Sci. USA* **92,** 4051–4055.

38. Pirrucello, S. J., Perry, G. A., Bock, P. J., Lang, M. S., Noel, S. M., Zon, G., and Iversen, P. L. (1994). HIV-1 *rev* antisense phosphorothioate oligonucleotide binding to human mononuclear cells is cell type specific and inducible. *Antisense Res. Dev.* **4,** 285–289.

39. Baeuerle, P. A., Rupec, R. A., and Pahl, H. L. (1996). Reactive oxygen intermediates as second messengers of a general pathogen response. *Pathol. Biol.* **44,** 29–35.

40. Driggers, W. J., Holmquist, G. P., LeDoux, S. P., and Wilson, G. L. (1997). Mapping frequencies of endogenous oxidative damage and the kinetic response to oxidative stress in a region of rat mtDNA. *Nucleic Acids Res.* **25,** 4362–4369.

41. Guidarelli, A., Brambilla, L., Rota, C., Tomasi, A., Cattabeni, F., and Cantoni, O. (1996). The respiratory-chain poison antimycin A promotes the formation of DNA single-strand breaks and reduces toxicity in U937 cells exposed to *t*-butylhydroperoxide. *Biochem. J.* **317,** 371–375.

42. Spencer, J. P. E., Jenner, A., Aruoma, O. I., Cross, C. E., Wu, R., and Halliwell, B. (1996). Oxidative DNA damage in human respiratory tract epithelial cells: Time course in relation to DNA strand breakage. *Biochem. Biophys. Res. Commun.* **224,** 17–22.

43. Collins, A. R., Dusinska, M., Gedik, C. M., and Stetina, R. (1996). Oxidative damage to DNA: Do we have a reliable biomarker? *Environ. Health Perspect. Suppl.* **104**(Suppl. 3), 465–469.

44. Petersen, S., Saretzki, G., and von Zqlinicki, T. (1998). Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. *Exp. Cell Res.* **239,** 152–160.

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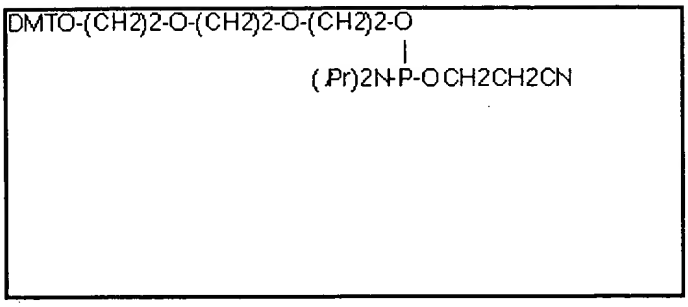
MATERIAL SAFETY DATA SHEET



SPACER PHOSPHORAMIDITE 9

SECTION I: GENERAL INFORMATION

CATALOG NUMBER(S): 10-1909-XX

PROPER NAME: 9-O-DIMETHOXYTRITYL-TRIETHYLENE GLYCOL,1-[(2-CYANOETHYL)-(N,N-DIISOPROPYL)]-PHOSPHORAMIDITE

COMMON NAME: SPACER PHOSPHORAMIDITE 9

CAS NO.: 146668-73-7

M.W.: 652.77

FORMULA: C36H49N2O7P

SECTION II: HAZARDOUS INGREDIENTS

Essentially 100%: 9-O-Dimethoxytrityl-triethylene glycol,1-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Contains no Metals, Pigments, Catalysts, Vehicle, Coating, Flux
TLV units: Unknown

SECTION III: PHYSICAL PROPERTIES

Appearance: Colorless to light yellow oil
Odor: None
Vapor Pressure: N/A
Boiling Point: Decomposes
Vapor Density: N/A
Percent Volatile by Volume : 0
Specific Gravity: ~1
Evaporation Rate (H2O=1): <<1
Solubility in H2O: Insoluble

SECTION IV: FIRE AND EXPLOSION HAZARD DATA

Flash Point: N/A
Extinguishing Media: Class A; Dry Chemical, CO2, H2O, Foam
Special Fire Fighting Procedures: None known
Unusual Fire and Explosion Hazards: None known

SECTION V: SPECIAL PROTECTION

Respiratory Protection: Local exhaust is usually sufficient.
Protective gloves: Impermeable.
Eye Protection: Laboratory safety glasses.
Other Protective Equipment: Normal laboratory apparel.

SECTION VI: Special PRECAUTIONS

Avoid all direct contact.

SECTION VII: REACTIVITY DATA

Stable Material, hazardous polymerization will not occur.
Decomposition Products:
-Toxic oxides of nitrogen may evolve when heated to decomposition.

SECTION VIII: HEALTH HAZARD DATA

Threshold limit value: Unknown.

Overexposure Effects: No data known.
*Material of unknown toxicity should be handled with care.
*All exposure should be minimized.

Emergency First Aid Procedures:
Always contact physician.
Ingestion:
*Contact physician.
*Never induce vomiting or give liquids to someone who is unconscious or cannot swallow.
Eye contact: Flush thoroughly with water.
Skin contact: Flush thoroughly with water.

SECTION IX: SPILL AND LEAKAGE PROCEDURES

If a spill occurs:
-Wipe with a damp towel and place material in a suitable container for disposal.

Waste Disposal: Comply with federal, state, and local regulations.

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